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Short communication

Acute ibogaine injection induces expression of the immediate early genes, *egr-1* and *c-fos*, in mouse brainSyed F. Ali ^{a,*}, Nathalie Thiriet ^b, Jean Zwiller ^b^a Neurochemistry Laboratory, Division of Neurotoxicology, HFT-132, National Center Toxicological Research, FDA, 3900 NCTR Road, Jefferson, AR 72079-9502, USA^b INSERM U338, Center de Neurochimie, Strasbourg, France

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Abstract

The aim of the present study was to evaluate if an acute injection of ibogaine (IBO) induces immediate early gene expression in different regions of mouse brain. Adult male C57 mice received a single injection of IBO and were perfused transcardially with 1% paraformaldehyde 30 min after the drug administration. A single injection of IBO produced a significant increase of *egr-1* messenger RNA induction in nucleus accumbens (NAc), caudate–putamen (CPu), frontal cortex (FCx), septum, dentate gyrus (DG) and CA3 region of hippocampus, whereas *c-fos* gene was induced in CPu, FCx, DG, septum and CA1 region of hippocampus. This gene expression may be due, in part, to the stimulant properties of IBO, as we found with other psychostimulants. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ibogaine; *Egr-1*; *C-fos*; Early gene expression; Neurotoxicity

Ibogaine (IBO), an indolalkylamine, is derived from the root of shrubs known as *Tabernathe iboga*, indigenous to West Central Africa. Earlier studies have reported that IBO and other similar indole alkaloids have hallucinogenic, as well as stimulant, properties (for review, see Popik et al. [25]). Recently, IBO has been proposed to end craving and withdrawal symptoms of psychostimulants such as cocaine and amphetamines, narcotics such as heroin, and even nicotine and alcohol in drug addicts. Animal studies have shown that IBO decreases cocaine consumption in a drinking preference model in mice [29], intravenous morphine self-administration, as well as dopamine release and locomotor activity induced by low doses of morphine in rats [11,12]. It has been shown that IBO binds with low micromolar potency to multiple neuronal targets; however, the role of the various binding sites in mediating specific actions of IBO is not clear. IBO has been shown to bind to dopamine and serotonin transporters, μ and κ opioid receptors, nicotinic receptors and to sigma-2 binding sites [13,20]. Some authors have speculated that the anti-addictive properties of IBO involve antagonist activity at

NMDA-type glutamate receptors, similar to MK-801 [17,31]. However, recent comparative neurobiological/neurochemical studies suggested that the mode of action of IBO is different from that of MK-801 [5]. We have also reported that a single injection of IBO produces significant changes in dopamine metabolism and neuroendocrine secretion [4] and neuropathological changes [27,28]. IBO was also shown to inhibit nitric oxide synthase [3], and to block methamphetamine-induced hyperthermia and induction of heat shock protein-72 [37].

We and others have reported that an acute injection of psychostimulants, such as cocaine or substituted amphetamines, induced immediate early gene expression in different brain regions [6,8–10,14,24,36]. Since these genes encode transcription factors, their activation is likely to play a key role in the transduction of short-lived environmental signals into long-lasting changes in the cell function. Activation of *c-fos* in the brain can be induced by a diverse group of stimuli (for review, see Herrera and Robertson [15]). The inducibility of *c-fos* can now be regarded as a tool to study neuronal activation in different systems in the brain. The immediate early gene, *egr-1*, has been shown to be involved in neuronal plasticity of hippocampus, especially in long-term potentiation [1,34] and in cognitive processes [22]. The present study was de-

* Corresponding author. Fax: +1-870-543-7745; e-mail: sali@nctr.fda.gov

signed to determine if an acute injection of IBO produces significant changes in *egr-1* and *c-fos* expression in mouse brain.

Adult male C57 mice from the NCTR breeding colony were used in this study. Animals were housed four per cage under controlled conditions. Water and food were provided ad libitum. The mice were injected with saline, or IBO, 50 mg/kg, i.p. ($n = 6-8$) and 30 min later, animals were given an overdose of pentobarbital (50 mg/kg, i.p.) and perfused transcardially with 0.9% saline (10 ml) followed by 1% paraformaldehyde in phosphate-buffered saline (pH 7.2; 50 ml). The brains were frozen in isopen-

tane at -40°C , and stored at -80°C . Coronal tissue sections (10 μm thick) were thaw-mounted onto gelatin-coated glass slides and store at -80°C .

In situ hybridization with riboprobe for *egr-1* and *c-fos* was performed as described previously by Thiriet et al. [32] with [^{35}S]uridine triphosphate (UTP)-labeled RNA probes. Briefly, sections were delipidated, acetylated, pre-hybridized for 10 min at 60°C in 50% formamide, $1 \times \text{SSC}$ (150 mM NaCl and 15 mM sodium citrate, pH 7.0), dehydrated and air-dried. Thirty microliters of the labeled probe, diluted to 60,000 dpm/ μl with hybridization buffer (50% formamide, $4 \times \text{SSC}$, 10% dextran sulfate and 10

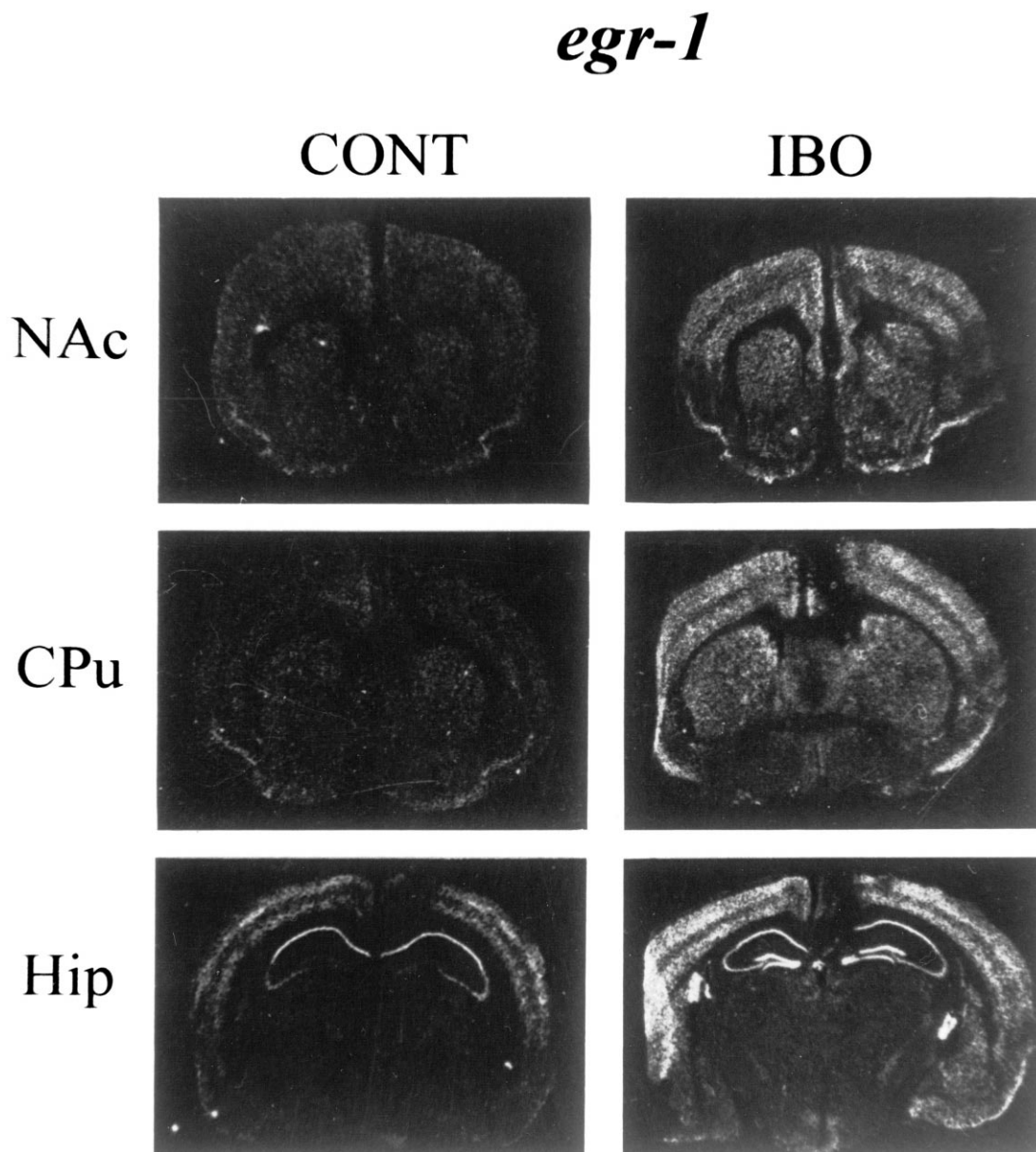


Fig. 1. Acute effect of IBO on *egr-1* induction in mouse brain. Negative prints of in situ autoradiograms showing *egr-1* expression. Coronal sections were hybridized with [^{35}S]*egr-1*-labeled antisense riboprobe. Mice were injected (i.p.) with saline (control) or with 50 mg/kg IBO and sacrificed 30 min later. Sections shown were taken at the level of NAc, approximately 4.7 mm anterior to interaural duct; of Cpu, approximately 3.7 mm anterior to interaural duct; and of Hip, approximately 1.0 mm anterior to interaural duct.

mM dithiothreitol), was placed on tissue sections and covered with coverslips. Hybridization was carried out overnight at 52°C. Hybridization medium was then washed off and the sections were washed twice in 50% formamide, $1 \times$ SSC at 55°C for 1 h, followed by two washes in $2 \times$ SSC (5 min, room temperature). Sections were incubated in a 10 mM Tris–HCl buffer (pH 8.0) containing 100 mM NaCl, 1 mM EDTA and 6.10^{-3} U/ml RnaseA (Merck) for 30 min at 37°C. The slides were then rinsed, dehydrated and exposed to X-ray film (Kodak Biomax-MR) for 2–4 days. To obtain quantitative results, densitometry was determined with a Biocom 2000 image analyzer and the

results expressed in kilobecquerel per gram tissue, using [14 C]microscales (Amersham) for calibration as described previously [16,32].

Acute injection of IBO produced the induction of immediate early genes, *egr-1* and *c-fos*, in selected regions of mouse brain. As shown in Fig. 1, in situ hybridization for *egr-1* showed a prominent high signal in caudate–putamen (CPu), frontal cortex (FCx), septal area, as well as in the CA3 and dentate gyrus (DG) subfields of hippocampus. The *egr-1* mRNA level was less induced in nucleus accumbens (NAc) and hippocampal CA1. Similar treatment of IBO increased *c-fos* mRNA expression in CPu, FCx,

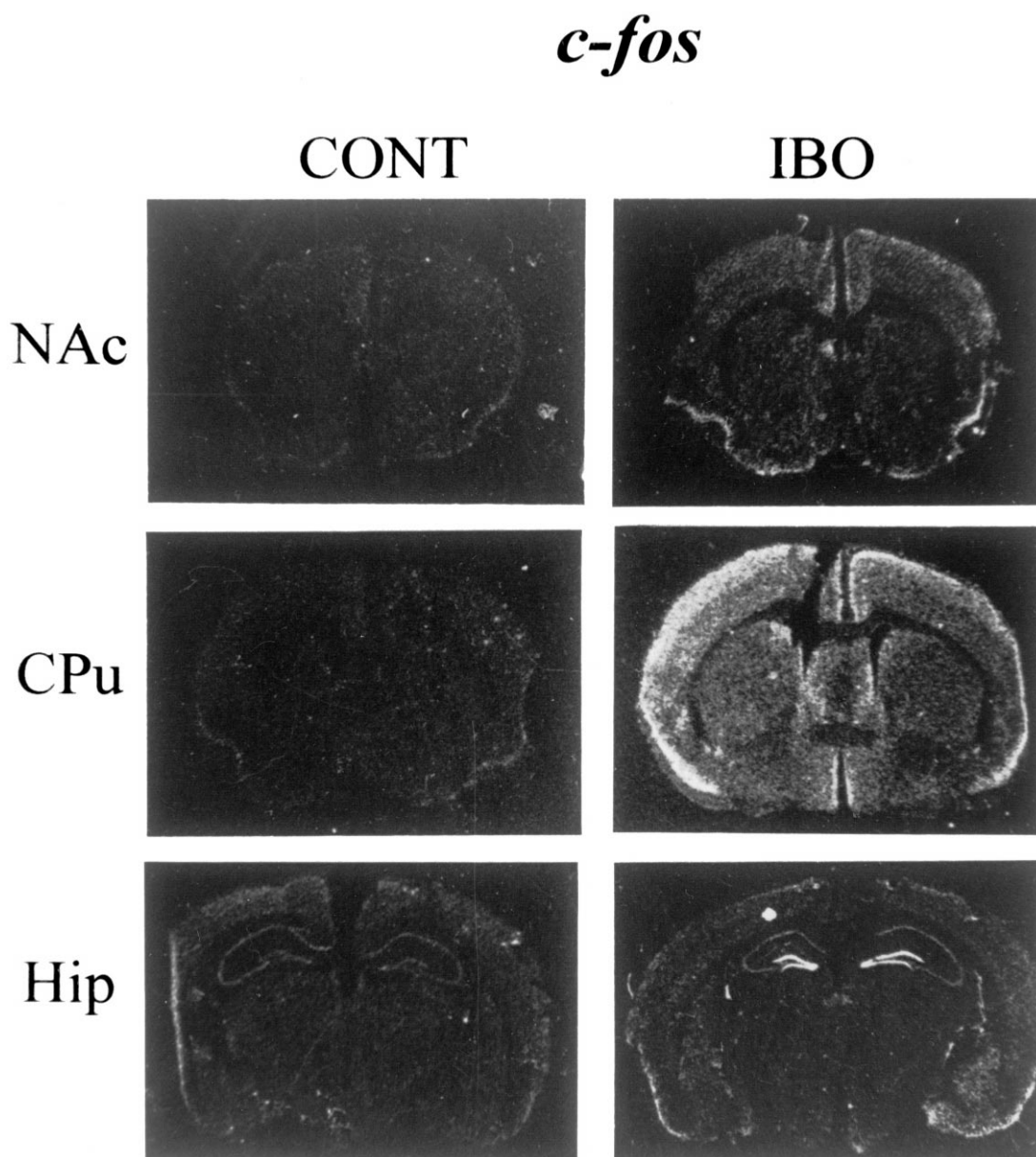


Fig. 2. Acute effect of IBO on *c-fos* induction in mouse brain. Negative prints of in situ autoradiograms showing *c-fos* expression. Coronal sections hybridized with [35 S]*c-fos*-labeled antisense riboprobe. Mice were injected (i.p.) with saline (control) or with 50 mg/kg IBO and sacrificed 30 min later. Sections shown were taken at the level of NAc, approximately 4.7 mm anterior to interaural duct; of Cpu, approximately 3.7 mm anterior to interaural duct; and of Hip, approximately 1.0 mm anterior to interaural duct.

Table 1

Effect of IBO on *c-fos* and *egr-1* gene expressionResults of densitometric analysis are expressed in kilobecquerel per gram tissue as mean $\pm$ S.D. ($n = 4$).

	<i>egr-1</i>		<i>c-fos</i>	
	Control	IBO	Control	IBO
NAc	5.78 $\pm$ 0.61	7.50 $\pm$ 0.37*	1.45 $\pm$ 0.13	1.95 $\pm$ 0.41
CPu	7.23 $\pm$ 0.26	12.4 $\pm$ 0.72**	1.15 $\pm$ 0.06	2.40 $\pm$ 0.50*
FCx	9.65 $\pm$ 0.75	17.4 $\pm$ 1.3**	1.23 $\pm$ 0.13	3.30 $\pm$ 0.14**
DG	3.87 $\pm$ 0.31	11.5 $\pm$ 0.81**	1.53 $\pm$ 0.29	6.65 $\pm$ 0.76**
CA1	6.50 $\pm$ 0.52	7.70 $\pm$ 0.52	1.60 $\pm$ 0.26	2.75 $\pm$ 0.26*
CA3	4.87 $\pm$ 0.40	9.10 $\pm$ 1.10*	1.78 $\pm$ 0.26	2.40 $\pm$ 0.67
Septum	3.90 $\pm$ 0.71	8.10 $\pm$ 0.83**	1.47 $\pm$ 0.07	5.27 $\pm$ 0.77**

* $p < 0.01$, ** $p < 0.001$, following Newmann–Keul's t -test after ANOVA.

and septum, as well as in DG and CA1 region of hippocampus (Fig. 2). No prominent induction of *egr-1* and *c-fos* was found in other regions of the brain. Quantitative analysis revealed that IBO induced statistically significant induction of *egr-1* and *c-fos* in those areas of the brain when compared to control (Table 1). It is noteworthy that induction of both genes was highest in DG (197% and 334% increase for *egr-1* and *c-fos*, respectively). Immediate early gene expression was only induced by about 30% in the NAc, an increase that did not reach statistical significance in the case of *c-fos* induction.

This is the first report showing that acute IBO injection induced the immediate early genes, *egr-1* and *c-fos*, in different regions of mouse brain. These results suggest that the early gene expression may be partially mediated by changes in the monoaminergic neurotransmissions. Several laboratories have reported that single or multiple injections of IBO to rodents produce alterations in the monoaminergic system that correlate with behavioral changes [13,18,19,29]. We and others have reported that stimulants like cocaine or amphetamines induce the early genes, *egr-1* and *c-fos*, as well as the transcription factors, AP-1 and NF κ B, in different brain regions [6,9,14,16]. IBO has been shown to bind to dopamine and serotonin transporters, with a higher affinity for the latter [20]. Since IBO-induced early gene expression in CPu, FCx and NAc is very comparable to the induction described in response to cocaine, the induction in these areas may be considered as resulting from increase in dopaminergic and/or serotonergic neurotransmissions. In NAc and CPu, it is probably serotonin, which is predominantly involved in the effect of IBO, since it was shown that IBO actually decreases extracellular dopamine in striatum and has no effect in the NAc, whereas it increases dialysate dopamine in the FCx [18].

Besides, we found a very substantial increase in the mRNA of both early genes in septal nuclei and in the hippocampus, especially in the DG. Interestingly, this has not been described for cocaine or amphetamine. Due to the complex pharmacology of IBO, one can only speculate

about which neurotransmitter to attribute the hippocampal induction. Induction in these brain areas may be the consequence of NMDA-type glutamate receptor blocking, since there are some reports which speculate that anti-addictive properties of IBO may be mediated by the antagonist activity at NMDA-coupled ion channels, similar to what was described for MK-801 [7,21,26]. This is an interesting and viable hypothesis because MK-801 is known to block the long-term neuroadaptive changes associated with acute and chronic administration of psychomotor stimulants, opioids or ethanol [1,2,23,33,35]. IBO also blocks depolarization evoked by NMDA in cultured hippocampal neurons and in intact motoneurons [7,21] and inhibits the binding of [3 H]MK-801 in brain membrane preparation [25,30]. On the other hand, serotonergic transmission is a likely candidate for being involved in hippocampal gene induction since (i) the hippocampus, particularly the DG, receives substantial serotonergic projections from the median raphe, and (ii) the hippocampal formation and the septal area are reciprocally connected. It is noteworthy that IBO-induced increased extracellular serotonin levels appears to be transient and may mediate the hallucinogenic manifestations reported during the early hours after IBO treatment in human [13]. However, it remains to be determined whether IBO's anti-addictive properties are related to its interaction with NMDA receptor or with the serotonergic system.

In summary, the present study indicates that acute injection of IBO can induce early gene expression in monoaminergic projections in a way similar, although not identical, to what was found for other psychostimulants. Further studies are needed to clarify the regional differences in the expression of these early genes induced by IBO and the role they play in the anti-addictive properties of IBO.

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